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Synthesis of 15 Amino Acid Gtp-Ase Binding Region of Tuberous Sclerosis 2 to Study its Interaction with the Ras Gtp-Ase Rheb

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Tuberous sclerosis 2 (TSC2) is a gene in the human body associated with benign tumorigenesis. TSC2 activates Rheb (Ras homology enriched in brain) protein, causing cell growth and production. An improper interaction of TSC2 with the Rheb protein has been shown to have these disease-causing effects. Rheb protein is a member of the Ras (Raus Sarcoma) family of proteins and is connected to cell growth, energy, and nutrient levels. The study of the improper interaction between TSC2 and Rheb has not been fully explored, and would lead to advances in treatment and prevention of this disease.

One obstacle in the study of the interaction between TSC2 and Rheb is the size of the TSC2 protein. TSC2 is an 1807 amino acid protein, which makes in-vitro characterization difficult. Thus, a smaller, representative region of the GTPase-binding domain of TSC2 must be synthesized in order for further exploration to take place. It has been determined that a 15 amino acid region of the TSC2 protein could be the site of the interaction with Rheb. In order to study the interaction of Rheb with this 15 amino acid peptide, the peptide was synthesized, along with several mutant peptides. Once these peptides were synthesized, their binding affinities for Rheb were measured using Isothermal Titration Calorimetry. In addition, a determination can be made as to each peptide's stabilizing effects on the TSC2-Rheb complex. Significant additions to the stability of this complex could lead to a decrease or elimination of the over-active Rheb signaling which leads to benign tumorigenesis.

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Time-Integrated Fluorescence Cumulant Analysis (TIFCA) Enables Model Selection in Ligand Binding Studies

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TIFCA is a fluorescence fluctuation spectroscopy technique that makes use of both the molecular brightness and diffusion time of freely diffusing fluorescent species. TIFCA was introduced to calculate statistically significant higher order cumulants to accurately describe protein interactions using rigorous fitting procedures. Previously, we have applied TIFCA to perform a titration, confirm antibody stoichiometry and measure the binding affinity by first assuming a bivalent antibody model. The cumulants were calculated up to forth order and globally fit to resolve the fraction of bound and unbound ligand. The global fitting routine uses all points in the titration simultaneously and returns a global reduced chi^2 for the entire titration series. It was found that analysis of the global reduced chi^2 alone is not sensitive to the chosen binding model. Here we show that TIFCA has the ability to determine proper binding models by evaluating fits to individual cumulants within the titration series. Using a small peptide and antibody, we show that local reduced chi^2 values are rather high for expressions describing a monovalent antibody. Only the correct number of species describing the bivalent nature of antibodies results in acceptable local reduced chi^2 values for all orders of cumulants. More specifically, it is found that the second order cumulant is highly dependent upon the number of binding sites in an antibody molecule leading to the correct binding model.

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Design of a Duet Expression Vector to Characterize Ligand-receptor Interactions

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One major challenge in the expression and purification of recombinant proteins is preventing proteins from forming inclusion bodies. Recovery of proteins from inclusion bodies can be laborious, and often a futile exercise. The partition between productive folding and non-specific aggregation/inclusion body formation is believed to be a competition between non-specific and specific structural interactions. Aggregation/inclusion body formation can be thwarted by promoting conditions that facilitate specific interactions during folding of the polypeptide chain.

D2 module of the extracellular immunoglobulin-like domain of the fibroblast growth factor (FGF) is overexpressed in *Escherichia coli* as inclusion bodies. Urea denaturation of inclusion bodies, and subsequent refolding, results in meager yields of the recombinant protein. In addition, the ''nativity'' of the refolded recombinant D2 module is always questionable. In this context, we have designed a novel Duet vector that co-expresses the D2 module and the ligand (FGF) simultaneously. It is predicted that the receptor module and FGF will form a soluble binary complex which can be dissociated under high salt conditions to simultaneously yield pure recombinant D2 module and FGF. Using the purified recombinant proteins, the structure of the FGF-receptor complex will be characterized using a variety of biophysical experiments, including multidimensional NMR spectroscopy.

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A Structural Based Design of a Novel Protein Affinity Tag for Purification of Recombinant Proteins

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Fibroblast growth factors (FGFs) are a family of heparin binding proteins that are involved in the regulation of key cellular processes such as cell proliferation, cell differentiation, wound healing, angiogenesis, and tumor growth. The present study is focused on the characterization of a novel heparin binding peptide (HEP-pep) designed based on the comparison of the available structures of FGF and other heparin binding proteins. Results of the isothermal titration calorimetry experiments show that HEP-pep binds to heparin with nanomolar affinity (K_d ~ 150 nM). Far UV CD spectrum of HEP-pep suggests that the peptide is predominantly in a random coil conformation. However, the peptide is observed to fold in to a helical conformation on binding to heparin. Steady state fluorescence experiments suggest that HEP-pep binds to heparin in a 1:1 stoichiometry. Conformational changes monitored by ^{1}H-^{15}N HSQC spectra of HEP-pep, in the absence and presence of heparin, shows a dramatic increase in the dispersion of the ^{1}H-^{15}N crosspeaks in the presence of heparin. Chemical shift index plot (based on H^{α} chemical shifts) shows that a conformational switch, from disordered state(s) to a helix, occurs in the HEP-pep on binding to heparin.

Based on the structural information on the HEP-pep, a recombinant vector was constructed and several target proteins with HEP-pep tag at N-terminus were overexpressed in high yields in *E.coli*. The HEP-pep fused target proteins were successfully purified to homogeneity in a single step on heparin sepharose. In addition, the HEP-pep tag does not appear to interfere with the folding of the target proteins. The details of the structure of the HEP-pep and its efficacy as an affinity tag for purification of recombinant proteins will be presented.

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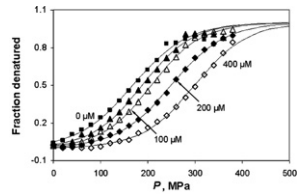
Determination of the Volume Changes Induced by Ligand Binding to Hsp90 Using High Pressure Denaturation

Daumantas Matulis.



The volume changes accompanying ligand binding to proteins are thermodynamically important and could be used in the design of compounds with specific binding properties. Measuring the volumetric properties could yield as much information, as the enthalpic properties of binding. Pressure-based methods are significantly more laborious than temperature methods and are underused. Here, we present a pressure shift assay (PressureFluor, analogous to the ThermoFluor, thermal shift assay) that uses high pressure to denature proteins. The PressureFluor method was used to study the ligand binding thermodynamics of heat shock protein 90 (Hsp90).

Increased concentration of a ligand caused the shift in the Pm as shown in the Figure. The volumes of the protein in the absence and presence of several ligands were determined. The compressibilities of the system could be also determined with significantly lower precision than the volumes. The formulas describing the relationship between the added ligand concentration and the Pm were derived and will be presented.

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Evolution of the Effect of Arginine on Thermal Stability and Oligomerization of N-Acetylglutamate Synthase

Ljubica Caldovic, Nantaporn Haskins, Amy Mumo, Mendel Tuchman,
Hiroki Morizono.

N-acetylglutamate synthase (NAGS; E.C.2.3.1.1) catalyzes the formation of N-acetylglutamate (NAG) from acetyl coenzyme A and glutamate. In microorganisms and plants, NAG is the first intermediate of arginine biosynthesis pathway, while in animals, NAG acts as an allosteric activator of carbamylphosphate synthetase I and III. NAGS itself is allosterically regulated by arginine. In bacteria, fungi, and plants, arginine acts as an inhibitor, in fish,